

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
29 January 2004 (29.01.2004)

PCT

(10) International Publication Number
WO 2004/009073 A1

(51) International Patent Classification⁷: **A61K 31/245**

In-Hyun [KR/KR]; 600-99, Sindaebang 1-Dong, Dong-jak-Gu, Seoul 156-011 (KR). YUK, Soon-Hong [KR/KR]; Hanbit Apt. 133-1105, Oeun-Dong, Yuseong-Gu, Daejeon 305-755 (KR).

(21) International Application Number:
PCT/KR2003/001441

(22) International Filing Date: 21 July 2003 (21.07.2003)

(74) Agent: PARK, Jang-Won; Jewoo Building 5th Floor, 200, Nonhyun-Dong, Gangnam-Gu, Seoul 135-010 (KR).

(25) Filing Language: Korean

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(26) Publication Language: English

(30) Priority Data:
10-2002-0042794 20 July 2002 (20.07.2002) KR

(71) Applicant (*for all designated States except US*): KOREA INSTITUTE OF SCIENCE AND TECHNOLOGY [KR/KR]; 39-1, Hawolgok-Dong, Sungbook-Gu, Seoul 136-791 (KR).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): CHUNG, Heson [KR/KR]; Ssangyong Apt. 3-507, Gwangyo-Dong, Nam-Gu, Incheon 402-715 (KR). JEONG, Seo-Young [KR/KR]; Munchonmaeul Life Apt. 205-501, Juyeop 2-Dong, Ilsan-Gu, Goyang, Gyeonggi-Do 411-747 (KR). KWON, Ick-Chan [KR/KR]; Siyoung Apt. 706-704, Hagye-Dong, Nowon-Gu, Seoul 139-230 (KR). PARK, Yeong-Taek [KR/KR]; Taeyoung Apt. 203-602, Bono 3-Dong, Ansan, Gyeonggi-Do 425-735 (KR). LEE,

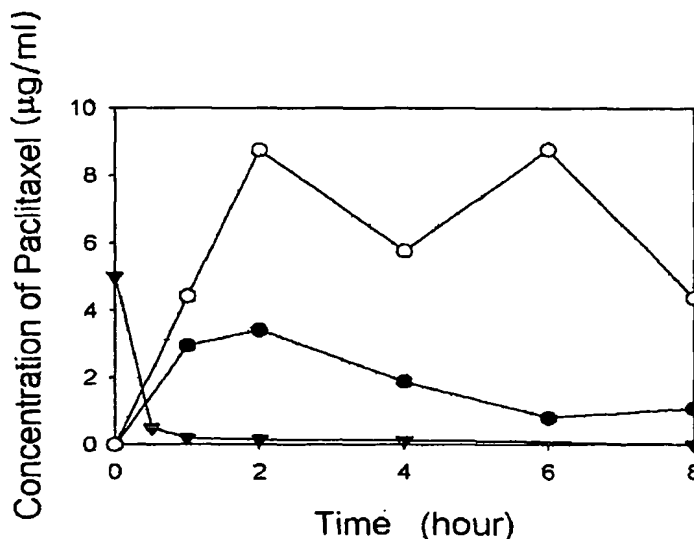
(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: P-GLYCOPROTEIN INHIBITOR COMPRISING OCTILONIUM BROMIDE AS AN EFFECTIVE INGREDIENT



(57) Abstract: The present invention relates to a new use of octylonium bromide as p-glycoprotein inhibitor to increase cellular uptake of drugs. More particularly, the present invention provides octylonium bromide as a p-glycoprotein inhibitor to increase cellular uptake of drugs such as anticancer drugs by taking octylonium bromide simultaneously with or preceding drug administration.

WO 2004/009073 A1

**P-GLYCOPROTEIN INHIBITOR COMPRISING OCTILONIUM BROMIDE
AS AN EFFECTIVE INGREDIENT**

[TECHNICAL FIELD]

5

The present invention relates to a new use of octylonium bromide to increase cellular uptake of drugs.

[BACKGROUND ART]

10

Octylonium bromide is an oral medication currently prescribed for the treatment of gastralgia and the irritable bowel syndrome. Octylonium bromide has an antispasmodic activity by loosening the contracted muscle in the stomach and the intestine resulted from hypersensitive reactions. Also, octylonium bromide is known as a medication of irritable bowel syndrome by controlling the motility and tension of the intestine. Since octylonium bromide is not absorbed into the intestine and acts mainly on the smooth muscle in the gastrointestinal tract, it does not have systemic side effects of anti-choline drugs including drowsiness and polydipsia. Octylonium bromide is also known as otilinium bromide or otilonium bromide.

20

P-Glycoprotein (pGP) is a product of multidrug resistance gene (MDR gene), and exists in the cell membrane to prevent the entrance of many toxic materials into the cytosol. P-Glycoprotein expels various absorbed toxic materials, especially anticancer drugs, out of the cell. Cancer cells that express p-glycoprotein do not respond well to the

25

anticancer drug treatment and, the drug resistance increases with repeated dose of anticancer drugs.

P-Glycoprotein is known to distribute in the blood-brain barrier (BBB) and mucous cells in the intestine. To overcome the anticancer drug resistance and to make the anticancer drug or other physiologically active compounds to be absorbed upon oral administration, therefore, it is essential to develop drugs that can inhibit the activity of p-glycoprotein. Many p-glycoprotein inhibitors are known up to date including cinchonin, calcium channel blockers such as verapamil and dihydropyridines (for instance nifedipine, nicardipine and nitrendipine), calmodulin antagonists such as trifluoroperazine, Vinca alkaloids such as vincristine and vinblastine, and immunosuppressants such as cyclosporine A. Among them verapamil used for irregular heartbeats or chest pain (angina) can cause nausea and gastroenteric disorder. Nifedipine, used to treat high blood pressure, can cause side-effects such as low blood pressure, dizziness, flushing (feeling of warmth), constipation and nausea. Vincristine and Vinblastine can also cause severe side-effects. Cyclosporine A, one of the most potent p-glycoprotein inhibitors, can cause harmful effects on the immune system since it is an immunosuppressant.

To overcome these problems, many p-glycoprotein inhibitors with lower side-effects and higher activity are being developed. A cyclosporin analog, SDZ PSC833, which does not lower the immune function was developed (Novartis). Indane derivative that can inhibit p-glycoprotein was also synthesized by Dr. Yoo in Korea Research Institute of Chemical Technology.

While searching for p-glycoprotein inhibitors, the present inventors have discovered that octylonium bromide, a calcium channel blocker, can help the absorption of drugs that are pumped out of the intestinal cells by p-glycoprotein. Octylonium bromide as the absorption enhancer in the present invention has a broader applicability and less toxicity than the absorption enhancer described in US 5,968,972. When administered orally, only 5 % of octylonium bromide is absorbed systematically, and the rest 95 % remains in the gastrointestinal tract. Since the local concentration in the intestine is higher, octylonium bromide can increase the oral bioavailability of the drugs effectively. The unabsorbed 95 % is discharged, and therefore minimizing the systemic toxicity. Also, the concentration of the anticancer drug in the blood increases with an increasing dose of octylonium bromide.

< Summary of the Invention >

The object of the present invention is to provide a novel use of octylonium bromide as an inhibitor of p-glycoprotein.

Another object of the present invention is to provide a novel use of octylonium bromide as an absorption enhancer of drugs that have a low oral bioavailability by co-administration.

Another object of the present invention is to provide a novel use of octylonium bromide as an absorption enhancer of drugs that have a low oral bioavailability by co-administration.

[DETAILED DESCRIPTION OF THE INVENTION]

The object of the present invention is to provide a novel use of octylonium bromide as absorption enhancer of anticancer drugs that exist in the mucous cells in the intestine.

In the present invention, octylonium bromide, as an effective
5 ingredient of a p-glycoprotein inhibitor, is effective at the dose of 0.01 mg/kg to 1 g/kg of body weight. When the dose of octylonium bromide is lower than 0.01 mg/kg, it cannot inhibit p-glycoprotein. When the dose exceeds 1 g/kg, however, gastroenteric disorder can be caused.

Also, the present invention provides a slow release formula of
10 octylonium bromide that can provide the p-glycoprotein inhibition activity for the duration of 12 hours. The slow release formula is prepared by preparing uniform sized seed granules with regular size, by coating the granules with octylonium bromide and by coating the exterior layer with polymer that can control the release rate of drug.

15 Octylonium bromide according to the present invention can be administered together with or 30 minutes prior to the administration of a drug.

In order to increase the availability of anticancer drugs, octylonium bromide can be co-administered with anticancer drugs via intravenous
20 injection, intramuscular injection, intratumoral injection, subcutaneous injection, oral administration, intravesical administration or intraperitoneal administration. Among them, oral administration is the most preferable. Octylonium bromide can be administered as a tablet or capsule.

The drugs that have enhances oral bioavailability when administered
25 with octylonium bromide include anticancer drugs such as doxorubicin,

daunorubicin, vinblastine, vincristine, actinomycin D, paclitaxel, teniposide and etoposide, immunosuppressants such as cyclosporin A and FK506, antihyperlipidemia such as lovastatin, antihistamines such as terfenadine, steroids such as aldosterone, hydrocortisone, cortisol, corticosterone and dexamethasone, dopamine antagonists such as domperidone, HIV protease inhibitors such as amprenavir, indinavir, nelfinavir, ritonavir and saquinavir, cardiac drugs such as digoxin and quinidine, antinausea drugs such as ondansetron, antidiarrhea medicines such as loperamide, gout preparations such as colchicine, antibiotics such as erythromycin, anthelmintics such as ivermectin, antituberculosis drugs such as rifampin and fluorescent chemicals such as rhodamine 123.

Above octylonium bromide can increase the absorption of drugs encapsulated in the oily solution solubilizing insoluble drugs. The above oily solution can include at least one monoglyceride, at least one oil and at least one emulsifier

The above monoglycerides are selected from a group consisting of one or more saturated or an unsaturated monoglycerides having 10 ~ 22 carbon atoms in the hydrocarbon chain. Monoglycerides is selected preferably from a group consisting of monoolein, monopalmitolein, monomyristolein, monoelaidin and monoerucin or from a group consisting of the mixture of monoglycerides semi-synthesized from triglycerides of vegetable or animal oil, and more preferably monoolein.

The above oil is selected preferably from a group consisting of triglycerides, iodinated oil, vegetable oil and animal oil.

The above triglycerides are selected from a group consisting of one

or more saturated or unsaturated triglycerides having 2 ~ 20 carbon atoms in the hydrocarbon chain. For instance, triacetin, tributyrin, tricaproin, tricaprylin, tricaprין or triolein can be used.

The above iodized oils include iodized poppy seed oil such as
5 Lipiodol, Ethiodol and iodized soybean oil.

The above vegetable oils include soybean oil, cottonseed oil, olive oil, poppyseed oil, linseed oil or sesame oil.

The above animal oils include squalane or squalene.

The emulsifier is preferred to select from the group consisting of a
10 phospholipid, a non-ionic surfactant, an anionic surfactant, a cationic surfactant, and bile acid.

The phospholipid is preferred to select from the group consisting of a phosphatidylcholine (PC) and its derivative, a phosphatidylethanolamine (PE) and its derivative, a phosphatidylserine (PS) and its derivative and a
15 polymeric lipid wherein a hydrophilic polymer is conjugated to the lipid headgroup.

The non-ionic surfactant is selected from the group consisting of a poloxamer (also known as Pluronic: polyoxyethylene-polyoxypropylene copolymer), a sorbitan ester (Span), a polyoxyethylene sorbitan (Tween)
20 and a polyoxyethylene ether (Brij).

The anionic surfactant is selected from the group consisting of a phosphatidylserine (PS) and its derivative, a phosphatidic acid (PA) and its derivative or sodium dodecyl sulfate (SDS).

The cationic surfactant is selected from the group consisting of 1,2-
25 dioleoyl-3-trimethylammonium propane (DOTAP),

dimethyldioctadecylammonium bromide (DDAB),
N-[1-(1,2-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA),
1,2-dioleyl-3-ethylphosphocholine (DOEPC) and
3 β -[N-[(N',N'-dimethylamino)ethan]carbamoyl]cholesterol (DC-Chol).

5 The bile acid is selected from the group consisting of cholic acid, its salt and derivatives; deoxycholic acid, its salt and derivatives; chenocholic acid, its salt and derivatives; and lithocholic acid, its salt and derivatives.

Other additives can be added to the above composition to be within 5% by weight (with respect to the total weight of the composition). For
10 instance, the composition can further comprise alcohol, polyol or Cremophor to improve the solubility of the drugs, tocopherol or tocopherol acetate to prevent oxidation, and fatty acid, fatty acid ester or fatty acid alcohol to increase drug absorption.

15

BRIEF DESCRIPTION OF THE DRAWINGS

20

Figure 1 is a graph showing the total concentration of paclitaxel and their metabolites in blood after oral administration of Taxol® of Bristol-Myers Squibb Company and octylonium bromide as an absorption enhancer. The quantitative analysis was performed by ELISA.

25

- ● - ; a group orally administered with Taxol® of Bristol-Myers Squibb Company (1 mg paclitaxel),
- ○ - ; a group orally administered with Taxol® of Bristol-Myers Squibb Company (1 mg paclitaxel) 30 minutes after administering orally 2 mg of octylonium bromide, and

- ▲ - ; a group intravenously administered with Taxol® of Bristol-Myers Squibb Company (10 µg paclitaxel).

Figure 2 is a graph showing the total concentration of paclitaxel and their metabolites in blood after oral administration of tricaprylin emulsion encapsulating paclitaxel and octylonium bromide as an absorption enhancer.
5 The quantitative analysis was performed by ELISA.

- ● - ; a group orally administered with tricaprylin emulsion encapsulating paclitaxel (1 mg paclitaxel), and
- ○ - ; a group orally administered with tricaprylin emulsion encapsulating paclitaxel (1 mg paclitaxel) 30 minutes after
10 administering orally 2 mg of octylonium bromide.

Figure 3 is a graph showing the total concentration of paclitaxel and their metabolites in blood after oral administration of oily solution containing paclitaxel and octylonium bromide as an absorption enhancer. The
15 quantitative analysis was performed by ELISA.

- ● - ; a group orally administered with oily solution containing paclitaxel (1 mg paclitaxel), and
- ○ - ; a group orally administered with oily solution containing paclitaxel (1 mg paclitaxel) 30 minutes after administering orally 2 mg
20 of octylonium bromide.

Figure 4 is a graph showing the total concentration of paclitaxel and their metabolites in blood after oral administration of oily solution containing paclitaxel and different amounts of octylonium bromide as an absorption enhancer. The quantitative analysis was performed by ELISA.

- ● - ; a group orally administered with oily solution containing
-
- 25

paclitaxel (1 mg paclitaxel),

- ○ - ; a group orally administered with oily solution containing paclitaxel (1 mg paclitaxel) and 0.5 mg of octylonium bromide,

- ▲ - ; a group orally administered with oily solution containing paclitaxel (1 mg paclitaxel) and 2 mg of octylonium bromide, and

- △ - ; a group orally administered with oily solution containing paclitaxel (1 mg paclitaxel) and 4 mg of octylonium bromide.

Figure 5 is a graph showing the concentration of absorbed rhodamine 123 into the intestine depending on the concentration of octylonium bromide by using everted sac.

□; a group treated with 10 µg/ml of rhodamine 123, and

■; a group treated with 10 µg/ml of rhodamine 123 and 200 µg/ml of octylonium bromide.

Figure 6 is a graph showing the concentration of absorbed doxorubicin into the intestine depending on the concentration of octylonium bromide by using everted sac.

□; a group treated with 50 µg/ml of doxorubicin, and

■; a group treated with 50 µg/ml of doxorubicin and 200 µg/ml of octylonium bromide.

Figure 7 is a graph showing the release rate of octylonium bromide from the sustained release granules containing octylonium bromide prepared by external coating with compositions containing Eudragit RS 100.

- ● - ; granules with an external layer coating by a composition containing 10 % of Eudragit RS 100, and

- ○ - ; granules with an external layer coating by a composition

containing 20 % of Eudragit RS 100.

Figure 8 is a graph showing the release rate of octylonium bromide from the sustained release particle containing octylonium bromide prepared by external coating with compositions containing Eudragit NE 30 D.

- 5 - ● - ; granules with an external layer coating by a composition containing 10 % of Eudragit NE 30 D, and
- ○ - ; granules with an external layer coating by a composition containing 20 % of Eudragit NE 30 D.

Figure 9 is a graph showing the release rate of octylonium bromide
10 from the sustained release particle containing octylonium bromide prepared by external coating with compositions containing 10 % of Eudragit RS 100 and different amounts of HPMC.

- ● - ; granules with an external layer coating by a composition additionally containing 10 % of HPMC,
- 15 - ○ - ; granules with an external layer coating by a composition additionally containing 20 % of HPMC,
- ▲ - ; granules with an external layer coating by a composition additionally containing 30 % of HPMC,
- △ - ; granules with an external layer coating by a composition
20 additionally containing 40 % of HPMC, and
- ■ - ; granules with an external layer coating by a composition additionally containing 50 % of HPMC.

[Best Mode for Carrying Out the Invention]

The following examples show that absorption of various drugs, whose absorption is inhibited by p-glycoprotein, is enhanced by oral administration of octylonium bromide. This invention is explained in more detail based on the following Examples but they should not be construed as
5 limiting the scope of this invention.

Example 1. Oral administration of Taxol®

① Oral administration

Taxol® was administered into Balb/C mouse (6 ~ 7 weeks old, female) fasted for 4 hours previously by using a gastric sonde. One hundred and sixty seven microliters of Taxol® of Bristol-Myers Squibb Company was mixed with 0.5 ml water. As a single administered group, the above solution containing paclitaxel (corresponding to 1 mg paclitaxel per mouse) was administered orally. Another group of mice, a co-administered group, was
15 orally administered with 2 mg octylonium bromide dissolved in 200 µl of phosphate buffered saline (PBS) and, after 30 minutes, 167 µl Taxol® of Bristol-Myers Squibb Company mixed with 0.5 ml water. Concentration of paclitaxel in blood was determined 1, 2, 4, 6 and 8 hours after the oral administration by collecting blood from the eyes.

As a control group, Taxol® of Bristol-Myers Squibb Company was administered intravenously into Balb/C mouse (6 ~ 7 weeks old, female). The concentration of paclitaxel in blood was determined up to 8 hours after the administration. After mixing 0.1 ml of Taxol® with 5.9 ml of water, 0.1 ml of the mixture corresponding to 10 µg of Taxol® was administered by bolus
25 injection through tail vein. Concentration of paclitaxel in blood was

determined 0.5, 1, 2, 4 and 8 hours after the oral administration by collecting blood from the eyes.

② Determination of total concentration of paclitaxel and its metabolites in blood (ELISA method)

5 The total concentration of paclitaxel and its metabolites in blood was determined by using Anti-taxane monoclonal kit (Model number 8A10) of Hawaii Biotech Company. Paclitaxel is known to be converted to 6- α -hydroxypaclitaxel and 3'-*p*-hydroxypaclitaxel by CYP2C8 and CYP3A4, respectively. Various metabolites including the primary metabolites of
10 paclitaxel exist in the blood. Anti-taxane monoclonal kit enables us to determine the concentration of paclitaxel and all of the metabolites containing taxane ring (Grothaus, G.P., Bignami, G.S., O'Malley, S., Harada, K.E., Byrnes, J.B., Waller, D.F., Raybould, T.J.G., McGuire, M.T. and Alvaro, B., Taxane-specific monoclonal antibodies: measurement of
15 taxol, baccatin III, and 'total taxanes' in *Taxus brevifolia* extracts by enzyme immunoassay. J. Nat. Prod. 58, pp. 1003-1014, 1995).

 The blood sample was serially diluted 4 times. Taxol-protein coating antigen (blue label) was diluted 100 times by phosphate buffered saline (PBS). After 100 μ l of the diluted antigen solution was put into each well of
20 the 96-well plate, the plate was incubated for 1 hour. After the plate was washed 4 times with TBST, it was blocked by adding PBS containing 1 % bovine serum albumin for 1 hour. After each well was washed continuously four times with TBST, 50 μ l of the serially diluted samples were put into each well. After diluting HBC Taxol Standard (RED label) serially with PBST, 50
25 μ l of the diluted standard solution was put into each well. Fifty microliters of

the second antibody solution prepared by mixing 4.5 ml PBST and 50 µl of anti-taxane rabbit antibody (green label) was added in each well. After the wells were washed four times with TBST, 100 µl of secondary antibody solution diluted 1000 times with PBST was added and incubated for one hour. After washing the wells four times with TBST, 200 µl of pNPP solution at 1 mg/ml was added in each well. After incubating the plate for 1 hour at room temperature, the absorbance was measured by using ELISA reader at 414 nm and compared with that at 690 nm for quantitative analysis.

③ Results

The changes in the paclitaxel concentration in blood with time are shown in Figure 1. When the bioavailability of paclitaxel upon bolus injection was set to 100 %, the relative bioavailability upon oral administration of paclitaxel was calculated by the following formula.

$$\text{Bioavailability (\%)} = \frac{\text{AUCoral}}{\text{AUCiv}} \times \frac{\text{DOSEiv}}{\text{DOSEoral}} \times 100$$

15

Wherein, AUCoral and AUCiv represent area under the curve after oral and intravenous administration, respectively, and DOSEoral and DOSEiv represent the paclitaxel dose for the oral and intravenous administration, respectively. The bioavailability upon oral administration of Taxol® when compared to the bolus injection was 6.5 % whereas the bioavailability upon co-administration of Taxol® and octylonium bromide was 22.8 %. Co-administration of octylonium increased the bioavailability of

20

Taxo®I by ca. 3.5 times.

Example 2. Oral administration of tricaprylin emulsion encapsulating paclitaxel

5 Viscous oily solution was prepared by mixing 1g tricaprylin, 0.2 g Tween 80 and 12 mg paclitaxel by warming at 40 °C and by sonicating in a bath type sonicator for complete solubilization. To the above oily solution, 4.85 ml of water was added and sonicated by using a probe type sonicator (High intensity ultrasonic processor, microprocessor control, 600-Watt
10 model) for 2 min to prepare tricaprylin emulsion encapsulating paclitaxel. Paclitaxel precipitation was not observed under polarized light microscope, and phase separation was not observed either.

 The tricaprylin emulsion encapsulating paclitaxel (1 mg paclitaxel per mouse) was administered into Balb/C mouse by using identical methods as
15 in Example 1 (Group received paclitaxel alone). Another group was administered with a solution containing 2 mg octylonium bromide in 200 µl of phosphate buffer solution followed by 500 µl of the tricaprylin emulsion encapsulating paclitaxel (1 mg paclitaxel per mouse) after a 30 min interval (Group received paclitaxel and octylonium bromide). Blood was collected 1,
20 2 and 4 h after the administration of the compositions, the concentration of paclitaxel in the blood collected from the eyes was determined. The total concentration of paclitaxel and its metabolites in blood analyzed by ELISA is shown in Figure 2.

 The bioavailability of the group received paclitaxel alone when
25 compared to the bolus injection in Example 1 (1 µg paclitaxel per mouse)

was 0.0 % indicating that paclitaxel was not absorbed at all. On the other hand, the bioavailability of the group received paclitaxel and octylonium bromide was 0.63 %.

5 **Example 3. Oral administration of oily solution encapsulating paclitaxel**

Viscous oily solution was prepared by mixing 1 g monoolein, 1 g tricaprylin and 0.4 g Tween 80 and by warming at 40 °C. Twenty four milligrams of paclitaxel was added into the oily solution and sonicated in a bath type sonicator for complete solubilization.

The oily solution encapsulating paclitaxel (1 mg paclitaxel per mouse) was administered into Balb/C mouse by using identical method as in Example 1 (Group received paclitaxel alone). Another group was administered with a solution containing 2 mg octylonium bromide in 200 µl of phosphate buffer solution followed by 100 µl of the oily solution encapsulating paclitaxel (1 mg paclitaxel per mouse) after a 30 min interval (Group received paclitaxel and octylonium bromide). Blood was collected 1, 2 and 4 h after the administration of the compositions, the concentration of paclitaxel in the blood collected from the eyes was determined. The total concentration of paclitaxel and its metabolites in blood analyzed by ELISA is shown in Figure 3.

The bioavailability of the group received paclitaxel alone when compared to the bolus injection in Example 1 (10 µg paclitaxel per mouse) was 1.0 % indicating that only a small amount of paclitaxel was absorbed. On the other hand, the bioavailability of the group received paclitaxel and

octylonium bromide was 21.4 %.

Example 4. Oral administration of oily solution encapsulating paclitaxel according to the dose of octylonium bromide

5 One hundred microliters of the oily solution encapsulating paclitaxel prepared in Example 3 (1 mg paclitaxel per mouse) was administered into Balb/C mouse by using identical methods as in Example 1 (Group received paclitaxel only), together with various concentrations of octylonium bromide. Groups of mice were administered with solutions each containing 0.5, 2 and
10 4 mg octylonium bromide in 200 μ l of phosphate buffer solution followed by 100 μ l of the oily solution encapsulating paclitaxel (1 mg paclitaxel per mouse) after a 30 min interval (Groups received paclitaxel and octylonium bromide).

Blood was collected 1, 2 and 4 h after the administration of the
15 compositions, the concentration of paclitaxel in the blood collected from the eyes was determined. The total concentration of paclitaxel and its metabolites in blood analyzed by ELISA is shown in Figure 4.

The bioavailability of the group received paclitaxel alone when compared to the bolus injection in Example 1 (10 μ g paclitaxel per
20 mouse) was 2.3 % indicating that only a small amount of paclitaxel was absorbed. On the other hand, the bioavailability of the group received paclitaxel and octylonium bromide increased with increasing dose of octylonium bromide as shown in Table 1.

Table 1

Amount of orally administered octylonium bromide (mg)	Bioavailability (%)
0	2.3
0.5	31.1
2	41.9
4	104.4

Example 5. Ex Vivo Absorption experiment of rhodamine 123 by using everted sac model

Oral absorption of rhodamine 123 is well-known to be inhibited by p-glycoprotein. Ileum portion of the intestine was taken out after sacrificing the SD rats. The ileum was cut into 2-cm tubes in length, and everted so as to expose the mucous intestinal tissue to the exterior of the tubes. After being tied both ends of the everted sac, the sac was immersed in 1 ml Krebs-Ringer buffer (KRB solution, pH 6.4) containing 10 μ g/ml rhodamine 123. The immersed sac was stored in an oxygen supplied, 37 °C incubator for 1 hour (Group treated with rhodamine alone).

Another group of everted sacs was stored as described above with the exception that sacs were immersed in 1 ml Krebs-Ringer buffer containing 10 μ g/ml rhodamine 123 and 10 μ g/ml octylonium bromide (Group treated with rhodamine and octylonium bromide). After one hour of the incubation, the sacs were put in 1 ml Krebs-Ringer buffer. The sacs were homogenized and centrifuged. Supernatant was obtained to analyze the concentration of rhodamine 123 by Fluorimetry as shown in Figure 5. The amount of the absorbed rhodamine 123 in the group treated with rhodamine alone was 0.95 μ g/g tissue whereas that in the group treated with rhodamine and octylonium bromide was 4.3 μ g/g tissue. These correspond

to 3 and 14.5 %, respectively, of the total applied amount. The results show that octylonium bromide helped increasing the amount of absorbed rhodamine by 4.5 folds.

5 **Example 6. Ex Vivo Absorption experiment of doxorubicin by using everted sac model**

Oral absorption of doxorubicin is well-known to be inhibited by p-glycoprotein. Everted sacs were used to perform the ex vivo absorption experiment by using the same method as in Example 5 excepting that 50
10 μg/ml doxorubicin was used instead of rhodamine 123.

The absorbed amount of the doxorubicin in the group treated with doxorubicin alone was 0.4 μg/g tissue whereas that in the group treated with doxorubicin and octylonium bromide was 11 μg/g tissue. These correspond to 0.12 and 3.3 %, respectively, of the total applied amount. The results
15 show that octylonium bromide helped increasing the amount of absorbed doxorubicin by 28 folds.

Example 7. Preparation of slow release granules of octylonium bromide

20 1. Preparation of uniform sized granules

To prepare granules with uniform size, 355 ~ 500 μm size sugar particles were used as seeds and coated with a composition consisting of 300 g sugar, 100g HPMC 2910, 700 g corn starch, 20 g PEG 6000, 1050 g water, 950 g acetone and 1050 g ethanol by using a coating machine. The
25 size of the prepared granules was 650 ~ 710 μm in diameter. The coating

conditions are shown in Table 2.

Table 2

Preheating :	20 min
Inlet air temperature	28 °C
Outlet air temperature	23 °C
Inlet air flow setting	30
Spray:	100 min
Spray nozzle diameter	2.5 mm
Atomizing air pressure	1.8 ~ 2.3 bar
Outlet air temperature	23 °C
Inlet air temperature	32 °C
Inlet air flow setting	30
Type of collecting plate	D
Air flow rate	20
Spray pressure increase rate	0.5 bar/5 flow rate
Flow rate increase rate	5 flow rate /5 min
Temperature increase rate	2 °C/5 min
Drying :	30 min
Inlet air temperature	40 °C
Outlet air temperature	32 °C
Inlet air flow setting	40

5 2. Coating of drug containing layer on the prepared granules

The prepared granules (200 g) were coated with a coating solution containing 26.7 g HPMC 2919, 200 g corn starch, 70 g octylonium bromide, 5.3 g PEG 6000, 700 g water, 350 g acetone and 500 g ethanol. The size of the prepared coated granules was 800 ~ 1000 μm in diameter and the yield was 90 %.

3. External layer coating to achieve slow release

To control the release rate of the drug, a composition containing polymetaacrylate compound, Eudragit RS or Eudragit NE was coated on the drug coated granules. The components and compositions of the Eudragit RS and Eudragit NE are shown in Table 3 and Table 4, respectively.

Table 3. Composition of Eudragit RS 100 coating

Components	Weight (g)	
	Eudragit® 7%*	Eudragit® 14%*
particle	800	800
Eudragit® RS 100	56	112
Methylene chloride	428	856
acetone	408	816
talc	56	112
Triethyl citrate	8.4	16.8
Total amount	1,756.4	2,712.8

10

Table 4. Composition of Eudragit NE 30 D coating

Components	Weight (g)	
	Eudragit® 10%*	Eudragit® 20%*
particle	800	800
Eudragit® NE 30 D	266	532
water	400	800
talc	56	112
Triethyl citrate	12	24
Total amount	1,756.4	2,268

Example 8. Drug release experiment from the slow release granules of octylonium bromide coated with Eudragit RS 100

The slow release granules of octylonium bromide coated with Eudragit RS 100 prepared in Example 7 was immersed in water to determine the amount of released octylonium bromide as a function of time (Figure 7). When the content of Eudragit was 7 %, 50 % of the drug was released in 4
5 hours. On the other hand, approximately 40 % of the drug was released in 10 hours when Eudragit content was 14 %. The results show that the release rate of the drug can be controlled.

**Example 9. Drug release experiment from the slow release
10 granules of octylonium bromide coated with Eudragit NE 30 D**

The slow release granules of octylonium bromide coated with Eudragit NE prepared in Example 7 was immersed in water to determine the amount of released octylonium bromide as a function of time (Figure 8). When the content of Eudragit was 10 %, 50 % of the drug was released in 2
15 hours. On the other hand, approximately 20 % of the drug was released in 10 hours when Eudragit content was 20 %. The results show that the release rate of the drug can be controlled.

**Example 10. Drug release experiment from the slow release
20 granules of octylonium bromide coated with Eudragit RS 100**

The granules were formulated by preparing granules by the same method as in above Example 7 and by coating the granules with the composition containing octylonium bromide. Slow release granules of octylonium bromide coated with Eudragit RS 100 prepared in Example 7
25 were immersed in water to determine the amount of released octylonium

bromide as a function of time (Figure 7). In order to coat slow release external layer, the composition for external layer coating was prepared by adding 10, 20, 30, 40, and 50 % by weight of hydroxy propyl methyl cellulose 2910 (HPMC 2910) with respect to the weight of Eudragit RS 100 into the
5 composition containing 7 % Eudragit RS 100. The prepared compositions were used for external layer coating. The results of the octylonium bromide release experiment are shown in Figure 9. The release rate became slower as the amount of HPMC increased.

10

[Industrial Applicability]

The present invention provides a method of using octylonium bromide to lower the activity of p-glycoprotein. Octylonium bromide increased dramatically the bioavailability of paclitaxel when administered orally. Also, it is shown in the present invention that octylonium bromide
15 inhibits the activity of p-glycoprotein by performing the rhodamine 123 absorption experiments. Therefore, octylonium bromide can be used to increase the bioavailability of various drugs that are pumped out by p-glycoprotein.

CLAIMS

1. A p-glycoprotein inhibitor containing octylonium bromide as an effective ingredient to increase the absorption of drugs into the cell.
2. The p-glycoprotein inhibitor containing octylonium bromide as an effective ingredient according to Claim 1, wherein the dose of octylonium bromide is in the range between 0.01 mg/kg body weight and 1 g/kg body weight.
3. The p-glycoprotein inhibitor containing octylonium bromide as an effective ingredient according to Claim 1, wherein octylonium bromide is formulated as a slow release formula to sustain the release of octylonium bromide up to 12 hours.
4. The p-glycoprotein inhibitor containing octylonium bromide as an effective ingredient according to Claim 3, wherein the above slow release formula is formulated by preparing uniform sized granules as a seed, by coating the granules with a composition containing octylonium bromide and by coating polymer that can control the release rate of the drug to form an external layer.
5. The p-glycoprotein inhibitor containing octylonium bromide as an effective ingredient according to Claim 1 that can be administered with other drugs simultaneously.
6. The p-glycoprotein inhibitor containing octylonium bromide as an effective ingredient according to Claim 1 that can be administered 5 ~ 60 minutes before administering other drugs.
7. The p-glycoprotein inhibitor containing octylonium bromide as an effective ingredient according to Claim 1, wherein the administration

route is selected from intravenous injection, intramuscular injection, intratumoral injection, subcutaneous injection, oral administration, intravesical administration or intraperitoneal administration.

- 5 8. The p-glycoprotein inhibitor containing octylonium bromide as an effective ingredient according to Claim 7, wherein the type of the formulation is tablet or capsule.
- 10 9. The p-glycoprotein inhibitor containing octylonium bromide as an effective ingredient according to Claim 1, wherein the above drugs are selected from the group containing doxorubicin, daunorubicin, vinblastine, vincristine, actinomycin D, paclitaxel, teniposide, etoposide, cyclosporin A, FK506, lovastatin, terfenadine, aldosterone, hydrocortisone, cortisol, corticosterone, dexamethasone, domperidone, amprenavir, indinavir, nelfinavir, ritonavir, saquinavir, digoxin, quinidine, ondansetron, loperamide, colchicine, erythromycin, 15 ivermectin, rifampin and rhodamine 123.
10. The p-glycoprotein inhibitor containing octylonium bromide as an effective ingredient according to Claim 1 that is used to increase the absorption of anticancer drugs.
- 20 11. The p-glycoprotein inhibitor containing octylonium bromide as an effective ingredient according to Claim 1 that can be administered with drugs encapsulated in the oily solution comprising at least one selected from monoglyceride, oil and emulsifier.

1/6

FIG. 1

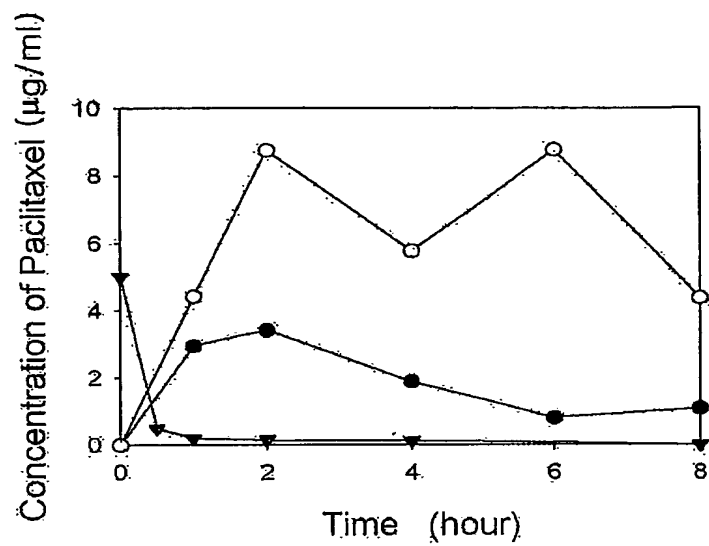
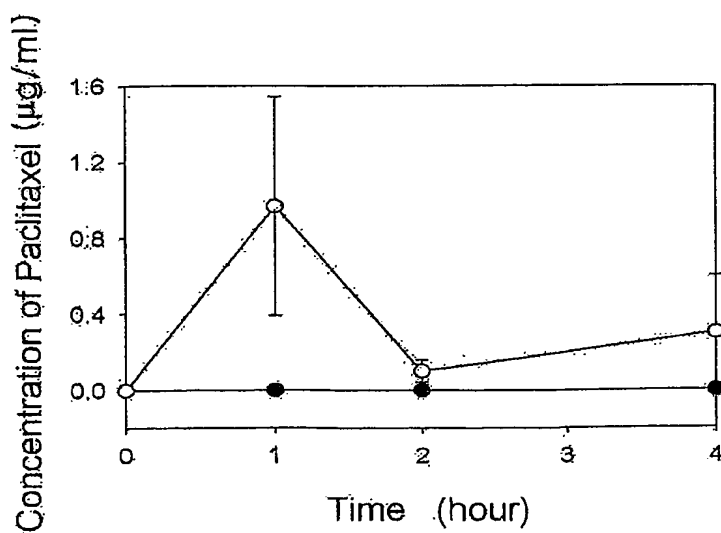


FIG. 2



2/6

FIG. 3

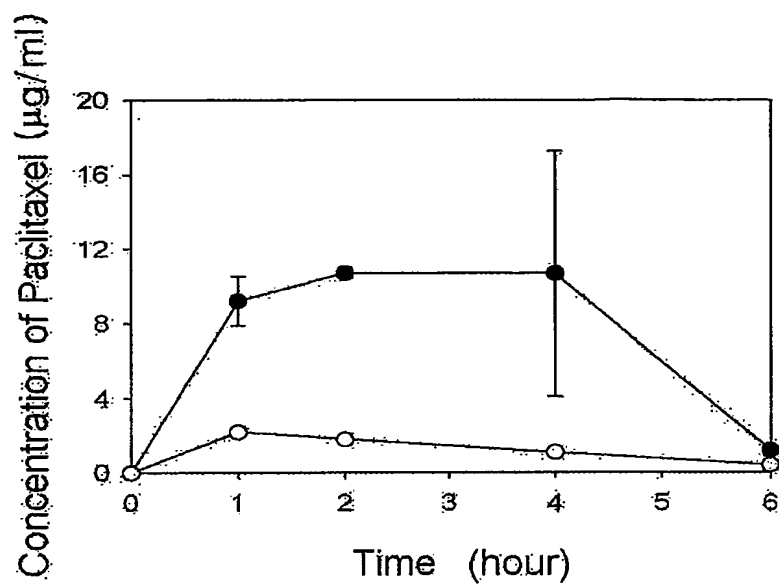
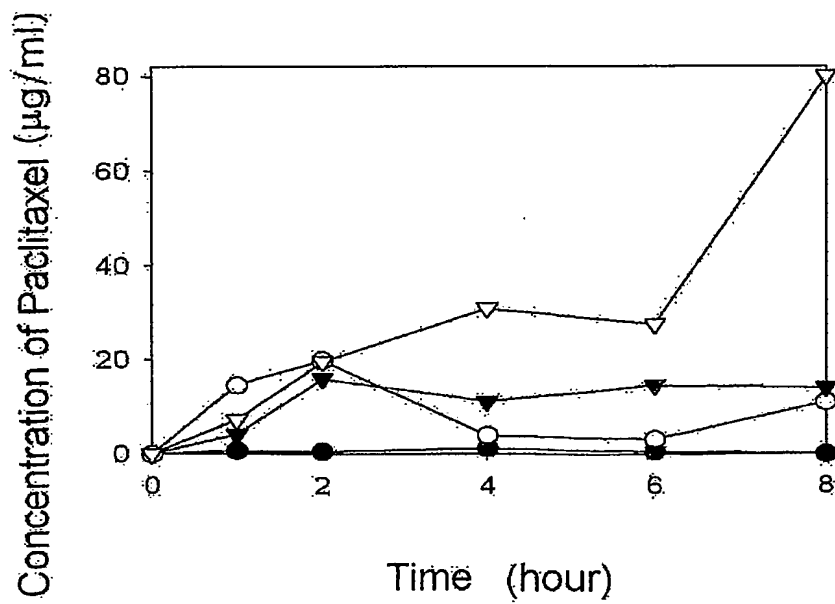
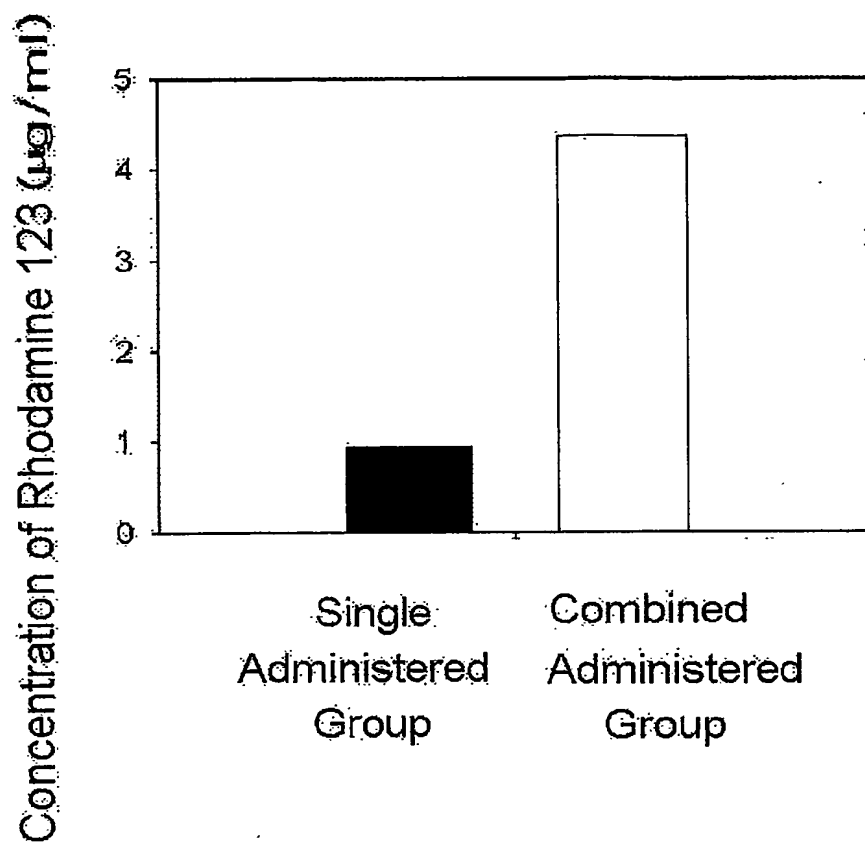


FIG. 4



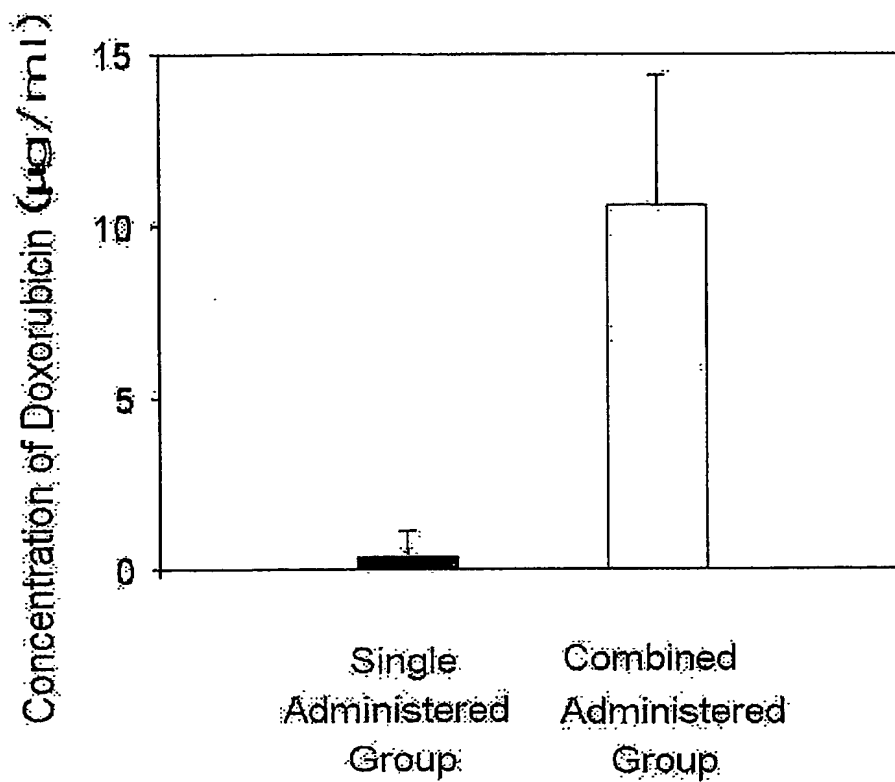
3/6

FIG. 5



4/6

FIG. 6



5/6

FIG. 7

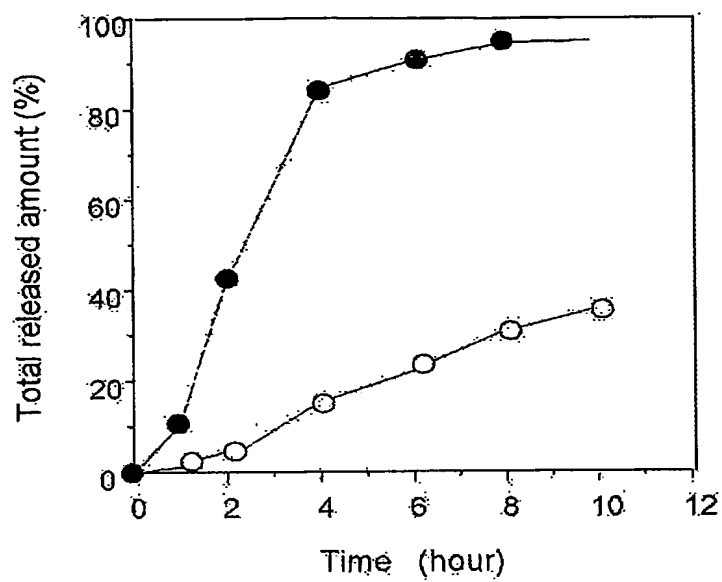
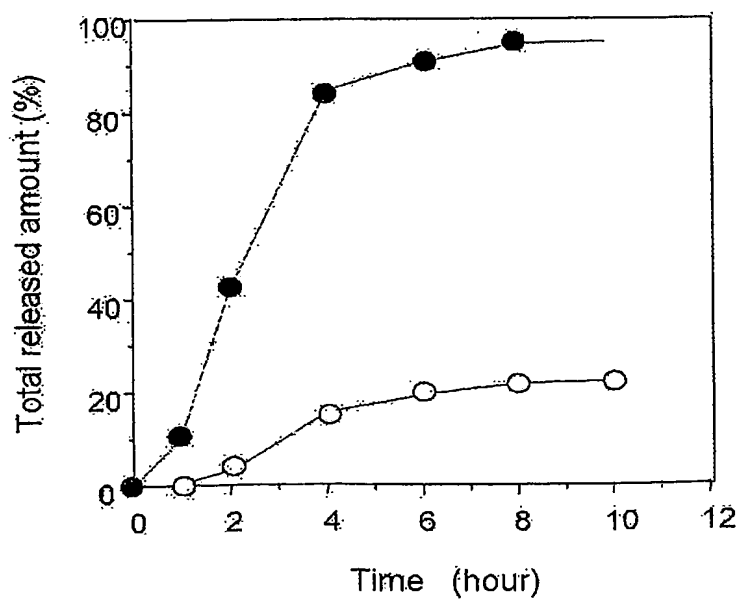
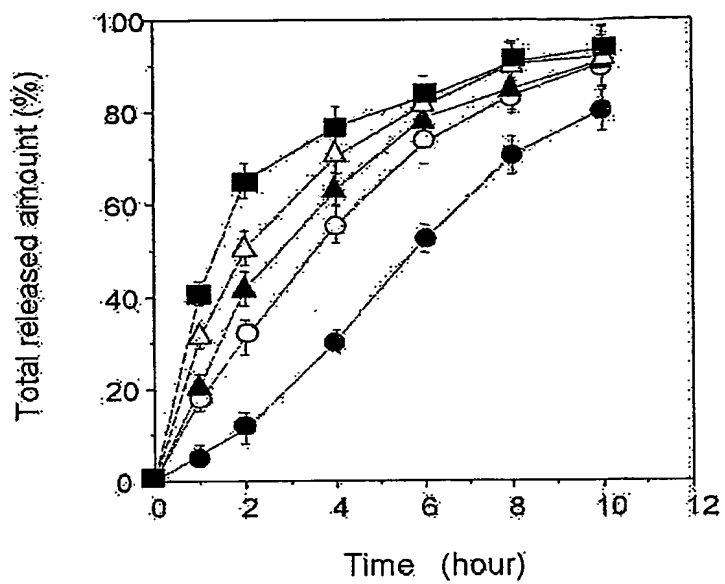


FIG. 8



6/6

FIG. 9



INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR03/01441

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 31/245

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K 31/245, C07C 229/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Patents and application for inventions since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MEDLINE, NPS, PAJ, CA on line, STN on line

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4978772 (A. MENARINI S.A.S.) 18 DEC 1990 see the whole document	1-11
A	Alimentary Pharmacology and Therapeutics, vol. 15, no. 3, pp. 355-361 (2001) see the whole document	1-11
A	Naunyn-Schmiedeberg's Archives of Pharmacology, vol. 359, no. 5, pp. 420-427 (1999) see the whole document	1-11
A	Pharmacological Research, vol. 38, no. 2, pp. 111-117 (1998) see the whole document	1-11
A	British Journal of Pharmacology, vol. 117, no. 3, pp. 463-470 (1996) see the whole document	1-11

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

27 OCTOBER 2003 (27.10.2003)

Date of mailing of the international search report

27 OCTOBER 2003 (27.10.2003)

Name and mailing address of the ISA/KR



Korean Intellectual Property Office
920 Dunsan-dong, Seo-gu, Daejeon 302-701,
Republic of Korea

Facsimile No. 82-42-472-3556

Authorized officer

BAIK, Kyong UP

Telephone No. 82-42-481-5600



INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR03/01441

Patent document
cited in search report

Publication
date

Patent family
member(s)

Publication
date

US 4978772

18. 12. 90

JP 2514670 B

10. 07. 96

IT 1216283 A

22. 02. 90

EP 0270503 B

03. 11. 87

DE 3787448 T

13. 01. 94

CA 1311419 A

15. 12. 92

AT 0094388 E

15. 10. 93